Effects of *Shewanella putrefaciens* Pdp11 on Senegalese sole skeletogenesis

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Introduction

*Solea senegalensis* is a highly valuable commercial fish. Skeletal abnormalities are a serious economical problem in sole aquaculture that may suppose about 15-40% of production discards. Probiotic supplementation has been reported to improve skeletal development in rainbow trout and sebass larviculture (Aubin et al., 2005; Lamari et al., 2013). *Shewanella putrefaciens* Pdp11 bioencapsulated in *Artemia* (10-86 dah) enhanced sole production parameters (Lobo et al., 2014). The aim of this study was to evaluate the effect of two different Pdp11 probiotic pulses using *Artemia* and rotifer (2-21 dah) or only *Artemia* (10-21 dah) as live vectors on the presence and severity of skeletal abnormalities in post-weaned (66 dah) sole juveniles.

Table 1: Skeletal areas affected (%) in *S. senegalensis* (66 dah) fed with the three experimental diets. (Mean ± SEM)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Cephalic vertebra</th>
<th>Prehernial vertebra</th>
<th>Hemal vertebra</th>
<th>Caudal vertebra</th>
<th>Caudal fin</th>
<th>Dorsal fin</th>
<th>Anal fin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCC</td>
<td>0.0 ± 0.0</td>
<td>12.6 ± 0.3</td>
<td>7.87 ± 1.40</td>
<td>58.5 ± 3.8</td>
<td>28.5 ± 3.3</td>
<td>57.8 ± 9.1</td>
<td>61.6 ± 2.9</td>
</tr>
<tr>
<td>CPC</td>
<td>18.1 ± 4.5</td>
<td>10.8 ± 2.2</td>
<td>13.3 ± 5.2</td>
<td>38.7 ± 10.0</td>
<td>33.1 ± 5.4</td>
<td>38.2 ± 4.2</td>
<td>33.5 ± 2.7</td>
</tr>
<tr>
<td>PPC</td>
<td>0.0 ± 0.0</td>
<td>8.33 ± 1.5</td>
<td>12.5 ± 2.6</td>
<td>38.7 ± 2.2</td>
<td>22.0 ± 4.1</td>
<td>25.6 ± 2.4</td>
<td>60.7 ± 0.6</td>
</tr>
</tbody>
</table>

Fig. 1a) Deformed CCC juvenile presenting a vertebral compression in the haemal vertebrae (HV) and an altered anal fin (c). b) Compression of haemal vertebrae 16-20 with partial fusion affecting haemal (HA) but not neural arches. c) Deformity in the anal fin caused by deformation of pterygiophores (Pt) that altering fin conformation but not fin rays (FR).

Fig. 2. a) Deformed CPC juvenile presenting a vertebral fusion and compression in haemal vertebrae (HV) 15-14. b) Fusion of caudal fin vertebrae (CFV) 43-44 with fused modified haemal arches (MHA) but not neural arches. Also present fused anal fin pterygiophores (Pt) and fused hypurals (Hyp). C) Fusion of haemal vertebrae 41-42.

Fig. 3. a) Deformed PPC juvenile presenting a deformity in the anal fin (AF) caused by deformed pterygiophores (Pt) and anal fin rays (FR) caused by fusion and misalignment of the pterygiophores (Pt) and anal fin rays (FR). b) Magnification of (b).

Discussion

Our results indicate a better skeletal condition in sole probiotic groups at the end of rearing, although the high variability observed confirm a further robust study. The average rate of deformities was similar to those described by Gavaia et al, (2009) and Bogliino et al, (2012), although the level of caudal and anal abnormal deformities was higher than those obtained by these authors. This findings may be due to a slight imbalance of minerals occurred at larval stages. The severity of the skeletal deformities detected, involving a loss of 12% of the fishes, was less than that observed in sole intensive larviculture (Bogliino et al., 2012).

Material and Methods

*S. senegalensis* larvae were distributed into 280 l tanks by triplicate. (19.3 ± 0.5 °C). Illumination and feeding regime was based on Lobo et al. (2014). Phytoplankton and rotifers were supplied (2-9 dah) and cofeeding was carried out with *Artemia* metanauplii (Orius and Sterleting) and dry feed (Larviva, Bimarc) since 10 dah. *S. putrefaciens* Pdp11 was daily incubated in TSA (1.5% NaCl) at 22°C, collected and suspended in a PBS solution (pH 7.2) and finally supplied to live feed (2.5±10³ cfu ml⁻¹) 3 hours prior to larval feeding. Three experimental groups were established: Control fish (CCC), CPC fish fed with Pdp11 (2-21 dah) and CPC fish fed with Pdp11 (10-21 dah). To identify and quantify different typologies of skeletal deformities in the 3 experimental groups 27 specimens per treatment were randomly sampled at 66 dah. Fish were anesthetised (triclofen, 40ppm) and fixed (4% formaldehyde in PBS, ph 7.4) for 24h and preserved (70% ethanol). For the visualization of the skeleton, tissues were submitted to specific staining procedures (alcian, blue for cartilage and alizarin red S for calcified structures) as Gavaia et al (2008). All observations were performed in a fluorescence stereomicroscopy Leica MZ7.5 equipped with a digital camera Olympus F-view. The deformities were separated according to the affected structures: cephalic (1-4), prehernial (5-9), hemal (10-42) and caudal (43-44) vertebra, caudal, dorsal and anal fin and cephalic area.

Results

Skeletal deformities registered in the three sole experimental groups were shown in Figure 4a, b. It was observed a great variability intragroup. No significant differences were detected among diets but probiotic groups revealed a decrease in deformed specimen of up to 11%. Severe deformities were below 12% in the 3 groups and were mainly located in the vertebral column (60%) and the anal fin (40%). No deformities were observed in the cephalic area. The most affected area in the vertebral column was the caudal vertebra (38.7±58.5 specimens affected) (Table 1). Skeletal deformities in cephalic, prehernial and hemal vertebrae did not exceed 20% in the three studied groups. Caudal, dorsal and anal fin abnormalities in the treatments ranged 22.0-33.1 %, 25.6-57.8 %, and 31.6-61.6 %, respectively (Table 1). Probiotic groups had a slightly higher incidence of fish without deformities (25.9% and 18.5% for CPC and PPC) compared to Control (7.4%) (Figure 4b). In this way Control group had a higher level of multiple abnormalities (48.2%) than probiotic groups (18.5-25.9%).

In conclusion: *S. putrefaciens* Pdp11 bioencapsulated in live diets seems to decrease skeletal deformities in sole fry. Our rearing regime seems to have a positive effect on skeletogenesis during sole rearing but further studies are needed providing higher number of samples to better establish the probiotic effects.

References


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